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(71) Applicant: UNIVERSITY OF SOUTHERN CALIFORNIA [US/US]; University Park, Los Angeles, CA 90007-4344 (US). (72) Inventors: DOUVAS, Angeline; 671 Delaware Drive, Claremont, CA 91711 (US). EHRESMANN, Glenn; 1941 Meadowbrook Road, Altadena, CA 91101 (US). TAKEHANA, Yoshi; 3500 Cedar Avenue #201, Long Beach, CA 90807 (US). (74) Agents: IMBRA, Richard, J. et al.; Campbell and Flores, Suite 700, 4370 La Jolla Village Drive, San Diego, CA 92122 (US).		Published <i>With international search report.</i>	
(54) Title: METHODS TO DIAGNOSE AND TREAT HIV-1 INFECTION			
(57) Abstract			
<p>The present invention provides a method for diagnosing HIV-1 infection in a subject by identifying the presence of anti-HIV-1 antibodies in the subject's serum that react with an autoantigen such as 70K. The invention also provides a method of stimulating an immune response against HIV-1 in a subject comprising immunizing the subject with an amino acid sequence of an autoantigen such as 70K that crossreacts with neutralizing epitopes present on HIV-1. The invention further provides a skin test that is useful for diagnosing a subject having an HIV-1 infection.</p>			

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METHODS TO DIAGNOSE AND TREAT HIV-1 INFECTION**BACKGROUND OF THE INVENTION****FIELD OF THE INVENTION**

The invention generally is related to the field
5 of immunology and more specifically to methods to diagnose
HIV-1 infection in a subject and to stimulate an immune
response against HIV-1.

BACKGROUND INFORMATION

As the incidence of acquired immune deficiency
10 syndrome (AIDS) continues to increase, the number of
persons infected by the type 1 human immunodeficiency virus
(HIV-1) has achieved an epidemic level. In spite of the
large amount of research aimed at elucidating the mechanism
and pathogenesis of AIDS, simple, inexpensive methods for
15 early diagnosis of HIV-1-infected individuals and methods
for immunizing a person against HIV-1 remain unrealized.

Upon infection of an individual with HIV-1, a
primary immune response is mounted against the virus. In
the primary response, a small number of lymphocytes have
20 the appropriate cell surface receptor to recognize the
virus. Upon recognition, the specific lymphocyte
population increases and begins secreting anti-HIV-1
antibodies into the circulation. Since only a small number
of lymphocytes can initially respond to the HIV-1
25 infection, a latent period occurs before circulating anti-
HIV-1 antibodies are present following infection. This
latent period lasts a minimum of one month and some persons
can remain seronegative for several months.

Following a primary immune response, specific memory cells remain in the circulation and, upon subsequent exposure to an antigen such as HIV-1, a secondary immune response is rapidly mounted. Antibodies generated in a 5 secondary immune response can be detected in the circulation within one or two weeks of exposure to HIV-1.

Two currently used tests for HIV-1 infection depend on the presence of circulating anti-HIV-1 antibodies in an infected individual. Both tests rely on the use of 10 an HIV-1-derived antigen, which is attached to a solid support. Although one of these tests is relatively simple and inexpensive to perform, it is only 90% specific. Thus, it can only be used as an initial screen and, if a positive result is obtained, the second test must be performed. A 15 positive result in the second test is diagnostic of HIV-1 infection. However, the second test is expensive and is laborious to perform. In addition, since the currently used tests rely on the detection of circulating anti-HIV-1 antibodies, they are inherently limited by the latent 20 period for generation of a primary immune response following HIV-1 infection. Thus, these assays cannot be used to detect HIV-1 infection within the first month after infection.

Efforts to provide immunologic protection against 25 HIV-1 infection also require viral protein. Various HIV-1 proteins have been used in an attempt to generate an anti-HIV-1 immune response. However, the use of viral material in humans carries the attendant risks associated with introducing such materials into a person. In any case, 30 these attempts at active immunization have been unsuccessful and decline of the immune system is an inexorable consequence of AIDS.

Thus, there exists a need for a simple, inexpensive diagnostic method that can identify an HIV-1-

infected individual at an early stage of infection and an effective method for stimulating an anti-HIV-1 immune response in a subject. The present invention satisfies this need and provides related advantages as well.

5

SUMMARY OF THE INVENTION

The present invention provides a method for diagnosing HIV-1 infection in a subject by identifying the presence of anti-HIV-1 antibodies in the subject's serum that react with an autoantigen such as 70K. The method of 10 diagnosis is particularly useful for diagnosing HIV-1 infection at an early time after a subject is infected.

The invention also provides a method of stimulating an immune response against HIV-1 in a subject comprising immunizing the subject with an amino acid 15 sequence of an autoantigen that crossreacts with neutralizing epitopes present on HIV-1. The invention provides, for example, amino acid sequences of 70K that are immunologically homologous to neutralizing epitopes of HIV-1, but not with regions of HIV-1 that mediate the 20 deleterious effects of the virus.

The invention further provides a skin test that is useful for diagnosing a subject having an HIV-1 infection. The skin test provides a simple, inexpensive method to screen large populations of persons suspected of 25 being infected with HIV-1.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the amino acid sequence homology between the U1 snRNP splicing protein 70K and gp120/41.

(A) Portions of the amino acid sequence of 70K 30 are shown in linear order in bold type (Theissen et al.,

EMBO J. 5:3209-3217 (1986), which is incorporated herein by reference). Numbers indicate amino acid positions from the amino terminus. Immunologically homologous sequences in gp120/41 and their amino acid positions appear above and 5 below, respectively, the sequence of 70K. The sequence boxed at amino acid position 322 in 70K indicates the consensus binding sequences (CBS). Underlined segments A and B indicate major epitope domains of 70K.

(B) Consensus binding sequences of a family of
10 U1 RNA binding proteins (Krainer et al., Cell 66:383-394
(1991), which is incorporated herein by reference) and the
immunologically homologous sequence in V3 of HIV-1 strain
IIIB. The solid box indicates the eight amino acid CBS;
15 broken boxes indicate the invariant amino acids, G and F,
and the nearly invariant amino acids, A and V. The CBS's
of heterogeneous nuclear ribonucleoproteins (hnRNP's) A2
and B1, which also are involved in splicing, are identical.
The nucleotide sequences of 70K and gp120/41 were obtained
from GenBank and translated into the amino acids shown
20 using a VAX/VMS computer.

Figure 2 illustrates the congruence of
neutralizing epitopes in the V3 loop of gp120 and
immunodominant epitopes in 70K. The amino acid sequence of
V3 strain IIIB (positions 303 to 338) was obtained from
25 GenBank. The numbered solid lines 1 to 7 represent
portions of the V3 sequence that reportedly induce
neutralizing antibodies. Regions of V3 that are
immunologically homologous to 70K are shaded. Internal to
V3 are shown the eight amino acids of the CBS of 70K (a),
30 U1 snRNP A and B1 (b) and hnRNP A1 (c). The CBS is within
the immunodominant B domain of 70K. Also shown is a
sequence spanning amino acid positions 239 to 248 of 70K,
including the immunodominant A domain, which is homologous
to amino acid positions 303 to 313 of V3.

Figure 3 compares the cross-reactivity between HIV-1 antigens gp120, V3 and gp41 and the anti-RNP antibodies as determined by ELISA. Sera were diluted 1:100 in phosphate-buffered saline/0.1% bovine serum albumin (PBS/BSA; pH 7.5). Horseradish peroxidase-conjugated goat anti-human antibody (Zymed, Inc.) was used as the second antibody and o-phenyldiamine dihydrochloride was used as the substrate. Optical densities (arbitrary units at OD₄₉₀) were recorded with an automated ELISA reader. Horizontal bars indicate the mean of each serum group.

Figure 4 compares the reactivity of anti-RNP and HIV-infected sera to V3 of HIV-1 strain IIIB (closed bars) and strain MN (open bars).

(A) HIV-infected sera 1 to 4 and a normal serum sample (NL);

(B) anti-RNP sera 1 to 7. Absolute OD₄₉₀ values varied by less than 5% between triplicates performed on the same day.

Figure 5 shows a western blot analysis of HIV-1-infected sera against U1 snRNP 70K. Partially purified 70K was isolated from rat liver nuclei by differential centrifugation and affinity chromatography on anti-RNP IgG-Sepharose as described by Douvas et al., J. Biol. Chem. 254:3608-3614 (1979), and Douvas, Proc. Natl. Acad. Sci., USA 79:5401-5405 (1982), each of which is incorporated herein by reference. Samples were fractionated by electrophoresis in a 10% polyacrylamide gel and transferred to nylon membranes for western blot analysis. HIV-infected sera, anti-RNP sera and normal sera were obtained as described in Figure 3 and diluted 1:250 for western blot analysis. Blots were developed using horseradish peroxidase-conjugated goat anti-human Ig (1:3000 dilution) as a second antibody (Tago). Lane 1 contains partially

purified U1 snRNP 70K antigen, showing 70K and a 60K breakdown product. Western blot strips were reacted with HIV-1-positive human sera (Lanes 2 to 11), with anti-RNP sera from MCTD patients (lanes 12 to 14), with no first 5 antibody (lane 15) or with control human sera (lanes 16 to 18).

Figure 6 shows the deduced amino acid sequence of the 70K polypeptide (Theissen et al., 1986).

DETAILED DESCRIPTION OF THE INVENTION

10 The present invention provides a method for diagnosing HIV-1 infection in a subject by identifying the presence of anti-HIV-1 antibodies that react with an autoantigen such as 70K, which is part of the U1 small nuclear ribonucleoprotein (snRNP) complex. The method is 15 particularly useful in that HIV-1 infection can be detected within one to two weeks of the time of initial HIV-1 infection.

As used herein, the term "70K" refers to a particular polypeptide that is a component of the U1 snRNP 20 complex. The deduced amino acid sequence of the entire 70K polypeptide is shown in Figure 6 (Theissen et al., EMBO J. 5:3209-3217 (1986), which is incorporated herein by reference; GenBank accession number X04654)). The term "70K" also is used more broadly to refer to an amino acid 25 sequence comprising a portion of the 70K polypeptide, provided that the amino acid sequence is immunologically homologous to the HIV-1 envelope glycoprotein, gp120/41 (see Figure 1.A.).

As used herein, the term "immunologically 30 homologous" means that either 1) two or more sequences of at least about ten amino acids have at least 50% amino acid identity or 2) two or more core sequences of at least about

four amino acids have at least 75% identity and, 3) in addition, identical amino acid sequences are present in the same order in each homolog, and 4) the amino acid sequence can crossreact with an anti-HIV-1 antibody. As used
5 herein, the term "core sequence" means a sequence of at least about four contiguous amino acids that are contained within a longer sequence. For example, the core sequence "GYAF" (SEQ ID NO: 22) is contained within the 70K consensus binding sequence "GYAFIEYE" (SEQ ID NO: 8). Such
10 amino-acid sequence homologies are considered significant because the probability of such matches occurring at random are 1 in 1.6×10^6 and 1 in 1.3×10^5 , respectively, and because the amino acid sequences crossreact with anti-HIV-1 antibodies. Examples of amino acid sequences of 70K that
15 are immunologically homologous to gp120/41 are provided in Figure 1. Amino acid sequences of other autoantigens such as the centromere protein, CENP-B, which is antigenic in scleroderma, also can be immunologically homologous to gp120/41 (Douvas and Sobelman, Proc. Natl. Acad. Sci., USA
20 88:6328-6332 (1991), which is incorporated herein by reference). Immunologically homologous amino acid sequences can be identified using the methods described herein.

The 70K polypeptide has a relatively hydrophobic
25 amino terminus and a hydrophilic carboxy terminus (Douvas and Sobelman, 1991). Three structures within the 70K polypeptide are important for antibody recognition. These three structures include the A and B domains as well as scattered hydrophilic sequences, including the consensus
30 ERPEEREERRR (SEQ ID NO: 23) sequence and the ERKRR (SEQ ID NO: 24) and RDRDR (SEQ ID NO: 16) motifs (see Figure 1.A.). Domain B encompasses a sequence of eight amino acids that is necessary and sufficient for binding to U1 RNA and is referred to herein as the "consensus binding sequence"
35 ("CBS") (see Surowy et al., Mol. Cell. Biol. 9:4179-4186 (1989), which is incorporated herein by reference).

70K is a component of the U1 small nuclear RNP nuclear RNA splicing complex, which also consists of a U1 RNA core and the associated polypeptides, A, B, B', C, D, D', F and G. The complex of U1 RNA and the 70K, A and C 5 polypeptides defines the RNP antigen, which is the target of IgG anti-RNP autoantibodies in the human systemic rheumatic disorder, mixed connective tissue disease (MCTD), and related syndromes (see below). 70K is the immunodominant polypeptide for anti-RNP autoantibodies and 10 is the target of autoimmune anti-RNP antibodies that are induced in MCTD.

As disclosed herein, autoimmune disorders constitute a paradigm for early HIV-1 infection. The autoimmune disorders such as MCTD, scleroderma and systemic 15 lupus erythematosus (SLE) belong to the class of systemic rheumatic diseases. A common characteristic of these disorders is the presence of T cell-dependent antibody production, wherein the antibodies react to the nuclear RNA splicing particle, U1 snRNP. In addition to reacting with 20 U1 snRNP, the anti-RNP antibodies crossreact with epitopes that are present on HIV-1 gp120/41 and are immunologically homologous to amino acid sequences of 70K. This crossreactivity forms a basis of the present invention.

70K shares multiple immunologically homologous 25 regions with the major neutralizing epitopes of gp120/41 (Table 1). These neutralizing epitopes also are the dominant sites for reactivity of anti-70K autoantibodies that occur in MCTD. One immunologically homologous region is shared between the functionally essential RNA binding 30 site of 70K and the apex of the V3 loop, which contains protease cleavage sites, which have a role in viral infectivity (see below and Figure 2). Another cluster of immunologically homologous regions involves the hydrophilic carboxy terminus of 70K and the epitopes in gp120/41 (see 35 Figure 1). One of these epitopes (sequence 4) is a peptide

Table I: Beneficial and deleterious epitopes in gp120/41; homologies to 70K and MHC determinants.

A. Neutralizing gp120/41 epitopes homologous to 70K				B. Deleterious and MHC-homologous epitopes			
gp120	Ref	gp41	Ref	gp120	Ref	gp41	Ref
1. 303 TRINNNTRKR (239) TRECERMLPKR	1, 38	4. 645 LIIESQNQEQEKN (80) LIEDQQQRQ	1, 3	579 RILAVERYLKNQQQLGIWCCSCKLIC	6		42
2. 311 RIQRGIGRAFTIG (98) PGRA	1, 3, 6, 33, 38,	5. 732 GPDPECGIEEGGGGERDRR (513) GPDGPDGPEEKGRDRDRR	3, 41	644 SLEQAQQQEKEKNEQELLKL	7		42
3. 469 RI.GGCCDMR (471) CCCDM	35	40. (408) (460) (542)	RDRDR DRDR RDRR	837 EGTDRLV (MIIIC II)	8	805 SDAKAYDTEV (MIIIC I)	11
(368) RLGGG (484) LRGGG				10. 56 GSTMGAAASMTLV (MIIIC I AND II)	9	this analysis	this analysis
				11. 527 QELKNSAVSL (MIIIC II)	10		

The HIV-1 sequences listed in panel A in bold type were identified as neutralizing epitopes in the published studies given as references by each sequence. Numbers to the left of each sequence indicate the position of the first amino acid in the sequence. 70K homologies appear below each HIV sequence, with a positions indicated in parentheses. Homologies were identified as described in Fig. 1. The deleterious and MHC-homologous epitopes in panel B were excerpted from the studies referenced beside each of the sequences 6-8. Additional MHC homologies in gp41 (sequences 9 and 11 in the table) and in gp120 (sequence 10) were identified as described in Materials and Methods.

sequence that strongly inhibits HIV-1 replication (Jiang et al., Nature 365:113 (1993)).

Although neutralizing antibodies are considered essential for immunoprotection against many viruses, their 5 role in HIV-1 infection is still ambiguous. Primary neutralizing determinants for anti-HIV-1 antibodies cluster in three regions of gp120/41: the V3 loop, the C4 domain and gp41 (see, for example, Moore and Ho, J. Virol. 67:863-875 (1993)). However, antibodies elicited in high titers by 10 vaccination with HIV-1 proteins may not target the most effectively neutralizing epitopes. Moreover, monoclonal antibodies acting in synergy can enhance neutralization or can enhance HIV infection. Adding further to the complexities of understanding and therapeutically 15 amplifying protective immunity is the role of discontinuous or conformationally sensitive epitopes, particularly in the C4 domain (see Moore and Ho, 1993).

As disclosed herein, IgG antibodies produced in patients suffering from the autoimmune disorder MCTD 20 crossreact with HIV-1 gp120/41. The primary antigen for MCTD patients is the RNA splicing protein, 70K, which shares immunologically homologous regions with gp120/41 (Figure 1). The cross-reactivity between anti-RNP 25 antibodies and gp120/41 is attributable to clusters of epitopes in V3 and gp41 homologous to 70K and appears to be sequence specific.

Autoimmune T cell clones and antibodies produced by B cells exist at low levels in normal individuals. In particular, a background level of anti-RNP antibodies can 30 be detected in normal individuals and these antibodies specifically react with 70K as shown by enzyme-linked immunosorbent assays (ELISA's) and western blot analysis (see Example II). Thus, normal individuals already have generated a primary immune response against 70K, this

response being analogous to having memory immune cells that are primed to generate a secondary immune response. These anti-70K memory immune cells can be stimulated by HIV-1 infection and, as a result, HIV-1 infection can be 5 diagnosed within one to two weeks following initial HIV-1 infection by detecting the presence of a greater than normal level of circulating anti-70 antibodies in a subject.

Immunologically homologous regions have been 10 identified between eight polypeptides, which are major antigens in the systemic rheumatic disorders, and several proteins involved in immune cluster viruses, including HIV-1, herpes simplex virus type 1 (HSV-1), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) (Douvas and Sobelman, 15 1991). However, only 70K and CENP-B have significantly more homology to the immune cluster viruses than do normal proteins. In fact, not only does 70K share extensive amino acid sequence homology (33%) with gp120/41, but there also is a congruence of dominant epitopes between the two 20 proteins that is strongly predictive of mutual antibody crossreactivity (see Figure 2). In contrast, 70K lacks the gp120/41 epitopes that are associated with deleterious effects, including enhancement of infectivity by antibodies, anergy, immunosuppression and accelerated 25 demise of CD4⁺ T cells (see Table 1.B.).

Cellular and humoral immune responses to HIV-1 that are neutralizing and, therefore, potentially protective, have multiple targets (epitopes) on gp120/41. Major neutralizing determinants for anti-HIV-1 antibodies 30 are found in a region of conserved and variable amino acid sequences in the V3 loop of gp120 (see Figure 2). The conserved sequences form potential proteolytic cleavage sites, including a trypsin site, GPGR ↓ AFVT, and a chymotrypsin-like site, GPGRAF ↓ VT. Cleavage at these 35 sites may be required for fusion of the viral and cellular

membranes and, therefore, for HIV infection of cells. Major neutralizing epitopes also have been identified in gp41, including some discrete regions involved in viral-host cell fusion and syncytium formation.

5 The epitope, GRAFVTIG (SEQ ID NO: 25), which is in the V3 loop of HIV-1 strain IIIB gp120 (V3 IIIB) is homologous to the functionally essential U1 RNA-binding site of 70K. Results obtained using ELISA assays revealed a mean reactivity of anti-RNP antibodies to V3 IIIB that is
10 as high as that of HIV sera (see Figure 3). Similarly, the V3 loop of HIV-1 strain MN contains the framework sequence GRAFXT (SEQ ID NO: 26; where "X" indicates any one independently selected amino acid) and also crossreacts with anti-RNP antibodies, as do hydrophilic epitopes in
15 gp41 homologous to the carboxy terminus of 70K. The GRAFXT (SEQ ID NO: 26) sequence also occurs in the V3 loop of HIV-1 strains SF2 and SC.

Strong crossreactivity between HIV sera and 70K also was observed using western blots (see Figure 5). In
20 contrast, antibodies from a related autoimmune disorder, Sjögren's syndrome (SS), are neither V3 nor gp41 selective. Thus, the substantial crossreactivity likely is due to conserved, antigenically dominant B cell epitopes having homologous counterparts in 70K and gp120/41.

25 The molecular mimicry and mutual crossreactivity between 70K and gp120/41 antigens and antibodies have significant functional, immunological and therapeutic implications. For example, amino acid positions 321 to 328 of the V3 loop and the CBS of 70K and similar U1 RNA-
30 binding proteins contain the conserved framework sequence that is immunologically homologous to the V3 loop of HIV-1 strains IIIB and MN GRAFVT (SEQ ID NO: 27) and GRAFYT (SEQ ID NO: 28), respectively, as delineated in Figure 1.B. Thus, the lack of marked strain specificity in anti-RNP

antibodies (Figure 4) can be attributed to their affinity for the conserved sequences.

The V3 sequence, GRAFVT (SEQ ID NO: 27), and its immunologically homologous forms in U1 RNA-binding proteins 5 are referred to collectively as the multifunctional (mf) motif because they are important in five different biological contexts: (1) the mf motif is a primary neutralizing determinant for antibodies in HIV infections; 10 (2) it is contained in the dominant epitope domain of 70K; (3) it has an essential role in RNA splicing (essentially all anti-RNP antibodies react with domain B of 70K, which contains the mf motif, and inhibit RNP splicing); (4) it contains proteolytic cleavage sites that may have an important function in viral and cell membrane fusion, and 15 therefore in HIV infectivity; and (5) it is an epitope not only for antibodies but also for T cells. Thus, the mf motif participates in interactions with an RNA molecule, an enzyme (protease), an IgG molecule and a T cell receptor.

The extended sequence, RIQRGPGRAFVTIG (SEQ ID NO: 20 29), the core of which is the mf motif, is an epitope domain for both CD4⁺ T helper (T_h) cells and CD8⁺ cytotoxic T lymphocytes (CTL) and can restimulate T cells that were previously exposed to HIV-1. These results, along with the 25 role of the mf motif in RNA splicing and the potent inhibition of splicing by anti-RNP antibodies were discussed above, indicate that the mf motif can be a common immunogen in both autoimmune disease and HIV infection.

The mf motif also can have a role in immunoregulation in these diseases. For example, the anti- 30 V3 and anti-70K titers of an MCTD patient that has been infected with HIV-1 for seven years fluctuate in tandem while anti-gp120 titers remain at high levels. In addition, the patient's CD4⁺ T cell counts fluctuate in parallel with the anti-V3/70K titers. Thus, the loss of

lymphocyte responsiveness to the mf motif may result in the demise of CD4⁺ lymphocytes. In contrast, perpetual responsiveness to this motif occurs in MCTD patients and results in sustained autoimmunity.

5 The results disclosed herein indicate that an autoimmune disease such as MCTD, scleroderma or SLE can be a useful model for developing immunoprotective strategies that allow sustained, high level immunity. The autoimmune model allows, for example, the development of an optimal
10 spectrum of antibodies. For example, in addition to the lack of specificity for deleterious HIV-1 epitopes (Table 1), anti-RNP antibodies are harmless in MCTD and, in fact, are correlated with a better clinical prognosis. Moreover, a significant number of anti-RNP sera inhibit syncytium
15 formation in HIV-1-infected target cells by greater than 90%, thereby demonstrating neutralizing potency. Also, 70K can be used as a surrogate immunogen for stimulating the immune system at both the B and T cell level. Furthermore, in addition to containing the mf motif, 70K also presents
20 multiple epitopes, which can act in concert to induce immunoresponsiveness.

The results provided herein also indicate that the autoimmune model can be useful for designing ligands based on U1 RNA and the mf motif. For example, a ligand
25 that binds to a functionally essential site on 70K also can be useful for binding immunologically homologous sites on V3. Such ligand binding can abrogate the role of V3 in infectivity. One target for such a ligand is U1 RNA, which contains an 8-10 nucleotide sequence that binds
30 specifically to the CBS of 70K. U1, but not U2, can bind to gp120 (not shown).

Because antibody production in both MCTD and HIV-1 infection is T cell-dependent, common T cell and B cell memory-like clones can be activated from a latent state in

these two diseases. The activation of these cells can result in both activation of latent cells and production of crossreacting anti-HIV-1 antibodies. The crossreacting antibodies and the presence of activated anti-70K latent 5 cells provide a method for diagnosing HIV-1 infection at an early stage. As used herein, the term "latent cell" means an autoimmune clone that is analogous to a memory cell, which is primed to react rapidly upon contact with a specific antigen.

10 A diagnostic test can be performed by contacting 70K or CENP-B with a sample obtained from a subject suspected of being infected with HIV-1 and, therefore, of having produced crossreacting antibodies. The sample can be, for example, a tissue sample or a sample of a body 15 fluid. The presence of anti-70K or anti-CENP-B antibodies in the serum can be determined using well known assays such as ELISA assays or western blots (see Example II). An autoantigen such as 70K can be obtained, for example, by extraction from an uninfected tissue or can chemically 20 synthesized or produced using recombinant DNA methods, as described below.

An autoantigen such as 70K can be attached to a solid substrate such as a plastic tissue culture well and anti-70K antibodies can be detected using an ELISA assay. 25 If 70K is obtained from tissue extracts, the 70K antigen may not be 100% pure. In this case, the preferred method of diagnosis is by western blot analysis, wherein 70K is fractionated by electrophoresis and transferred to a paper or nylon support. The western blot assay allows for 30 specific reactivity of an anti-70K antibody with a polypeptide that migrates at the expected molecular mass of 70K can be identified. On the otherhand, if recombinant 70K is used as an antigen, an ELISA assay can provide sufficiently precise diagnosis.

Early diagnosis of HIV-1 infection is desirable for many reasons. In particular, the disclosed method for early diagnosis of HIV-1 infection is useful for screening blood samples. The use of the disclosed method can 5 identify blood samples that are obtained from a donor that was infected with HIV-1 within the prior one to two weeks of donating the blood. Other potential methods of identifying HIV-1 infected blood at this stage of infection are prohibitively expensive and, therefore, are not used as 10 a matter of routine screening.

It can be desirable to provide a kit for performing the disclosed method of diagnosis. Such a kit can contain 70K attached to a solid support and also can contain, if desired, standard reagents such as a 15 predetermined amount of an anti-70K antibody. Such reagents can provide a means to readily determine whether a sample obtained from a subject contains a greater than normal amount of circulating anti-70K antibody. It is recognized that a population of normal serum samples must 20 be analyzed in order to determine the "normal" level of anti-70K antibody in a person that is not infected with HIV-1. However, methods to obtain a statistically significant normal level of anti-70K antibodies are well known and routine in the art.

25 The invention also provides a method of stimulating an immune response against HIV-1 in a subject comprising immunizing the subject with an amino acid sequence of an autoantigen such as 70K that is a surrogate for neutralizing epitopes present on HIV-1. Amino acid 30 sequences of 70K that cross-stimulate a protective or neutralizing immune response against HIV-1 can be identified by the immunologically homologous regions shared between these sequences in 70K and gp120/41. Examples of such amino acid sequences are provided in Figures 1 and 2. 35 Crossreactive and, therefore, cross-stimulating amino acid

sequences can be identified using methods such as ELISA and western blot analysis as described herein (see Example II).

Since autoimmune T cell clones and anti-70K antibodies produced by B cells exist at low levels in 5 normal individuals, these normal individuals have latent immune cells that are primed to generate a secondary immune response. It follows that 70K can be used as a surrogate immunogen that is useful as a vaccine to provide continuous stimulation of the immune system. As used herein, the term 10 "surrogate immunogen" means an autoantigen such as 70K or an amino acid sequence of an autoantigen that is immunologically homologous to a neutralizing epitope present on gp120/41 and that can stimulate an immune response in a subject against HIV-1. Thus, a surrogate 15 immunogen is an amino acid sequence that is immunologically homologous to HIV-1 and that, in addition, can stimulate an immune response.

A surrogate immunogen can be immunogenic by itself or can be attached to a carrier molecule such as 20 bovine serum albumen or an inert carrier such that the surrogate immunogen-carrier complex can stimulate an immune response. An immune response can be stimulated *in vivo* or *ex vivo*. For example, immune cells such as T cells and B cells can be obtained from a subject and placed in a tissue 25 culture medium. The cells can be contacted with a surrogate immunogen, which can stimulate the immune cells by inducing a primary or secondary immune response.

Anti-RNP antibodies are crossreactive with gp120/41 and are effective in arresting the infectivity of 30 HIV-1 in infected cells *in vitro* (data not shown). Specifically, the anti-RNP antibodies can recognize the GRAFTVIG (SEQ ID NO: 25) sequence. This result indicates that a surrogate immunogen such as 70K or GRAFTVIG (SEQ ID NO: 25), for example, can be used to stimulate memory anti-

70K cells in a subject and the anti-70K antibodies can crossreact with homologous epitopes present on gp120/41.

Use of a surrogate immunogen such as 70K is advantageous because it is an autoimmune protein that
5 represents an enrichment of sequences that can stimulate the early shared clones. As used herein, the term "shared clones" means anti-70K latent T cells and B cells that can be rapidly activated to react with HIV-1 or can produce crossreactive anti-gp120/41 antibodies that can neutralize
10 HIV-1. The use of a surrogate immunogen also is advantageous in that it can provide co-amplification or synergistic amplification of shared clones with gp120/41. In addition, a surrogate immunogen such as 70K does not contain amino acid sequences that stimulate "harmful"
15 antibodies that mediate the deleterious effects associated with HIV-1 infection (see Table 1). Furthermore, the use of a surrogate immunogen such as 70K precludes the introduction of viral-derived material into a subject.

An immune response against HIV-1 can be
20 stimulated in a subject by administering a therapeutically effective amount of a surrogate immunogen, which comprises an amino acid sequence of an autoantigen such as 70K that crossreacts with neutralizing epitopes present on gp120/41 and stimulates an immune response, and a pharmacologically
25 acceptable carrier. Pharmaceutically acceptable carriers are well known in the art and include aqueous solutions such as physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters. As used herein, the term
30 "therapeutically effective amount" means an amount of a surrogate immunogen that can stimulate an immune response. The amount will vary, of course, depending, for example, on whether stimulation of the immune response is *in vivo* or *ex vivo* or on whether the administration is a first
35 administration or a booster administration. A

therapeutically effective amount can be determined using methods known in the art (see, for example, Harlow and Lane, 1988).

A composition comprising a surrogate immunogen 5 and a pharmaceutically acceptable carrier also can contain an adjuvant if desired. Adjuvants, which include, for example, Freund's complete or incomplete adjuvant, are known in the art and commercially available (Ribi Immunochem Research, Inc.; Hamilton, MT). The addition of 10 an adjuvant can affect the amount of surrogate immunogen that is required to obtain a therapeutically effective amount.

A pharmaceutically acceptable carrier also can contain other physiologically acceptable compounds that 15 act, for example, to stabilize the surrogate immunogen or increase the absorption of the surrogate immunogen. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, 20 chelating agents, low molecular weight proteins or other stabilizers or excipients. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration of the 25 composition and on the particular physico-chemical characteristics of the surrogate immunogen. Various routes of administration are known in the art and include, for example, intravenous, intradermal and subcutaneous injection, oral administration and transdermal 30 administration.

The amino acid sequences of an autoantigen such as 70K that crossreact with neutralizing antibodies can be obtained, for example, by chemical synthesis of the amino acid sequences. A particularly useful means for obtaining

sufficient amounts of an amino acid sequence such as a peptide is by the use of recombinant DNA methods, which are well known in the art (see, for example, Sambrook et al., Molecular Cloning: A laboratory manual (Cold Spring Harbor 5 Laboratory Press 1989), which is incorporated herein by reference). For example, the polymerase chain reaction (PCR) can be used to amplify the nucleotides encoding an amino acid sequence of 70K that is immunologically homologous to gp120/41 and the amplified sequence can be 10 cloned into an expression vector, which allows for transcription and translation of the cloned sequence. The amino acid sequence then can be isolated in relatively pure form. Methods for amplifying a nucleotide sequence and cloning and expressing the nucleotide sequence are well 15 known in the art (see, for example, Sambrook et al., 1989; see, also, Ehrlich, PCR Technology: Principles and Application for DNA Amplification (Stockton Press 1989), which is incorporated herein by reference).

Methods for stimulating an immune response in a 20 subject are well known in the art and described, for example, in Harlow and Lane, Antibodies: A laboratory manual (Cold Spring Harbor Laboratory Press 1988), which is incorporated herein by reference. For example, the composition can be administered intradermally, 25 intramuscularly or intravenously. In addition, it can be advantageous to administer one or more booster immunizations. The need to administer a booster immunization can be determined experimentally by measuring the presence of anti-70K antibodies in a subject's serum 30 using the methods described herein.

The invention also provides a skin test that is useful for diagnosing a subject having an HIV-1 infection. A composition comprising an amino acid sequence that is immunologically homologous to an epitope present on HIV-1 35 and a pharmacologically acceptable carrier is administered

intradermally to a subject suspected of being infected with HIV-1. A diagnosis of HIV-1 infection is made by observing evidence of an immune response at the site of intradermal injection. Such evidence, which includes redness or 5 swelling at the site of injection, is indicative of a delayed-type hypersensitivity response, which, in turn, provides a positive diagnosis of HIV-1 infection.

The diagnostic skin test is performed by intradermal injection of about 0.1 ml of a composition 10 comprising a surrogate immunogen, which is an amino acid sequence that is immunologically homologous to an epitope present on HIV-1 and can stimulate an immune response, and a pharmacologically acceptable carrier (see Example III). The test results are evaluated by measuring the maximum 15 extent of erythema and induration or, if desired, by biopsy. The test is particularly useful in that the subject being tested can examine the site and, in the case of a negative result, can avoid a return visit to the health care provider for evaluation of the test result.

20 The following examples are intended to illustrate but not limit the invention.

EXAMPLE I

Immunologically Homologous Regions of 70K and HIV-1 gp120/41

25 This example demonstrates the high degree of homology shared between the immunodominant regions of 70K and the neutralizing epitopes of gp120/41 and identifies amino acid sequences of 70K that are immunologically homologous to HIV-1.

30 The HIV-1 strain IIIB DNA sequence (K03455) was obtained from GenBank and translated into the amino acid sequences for gp120 (amino acid positions 1 to 511) and gp41 (512 to 856). Sequences for V3 IIIB and V3 MN were

obtained from D'Souza et al., AIDS 5:1061-1070 (1991), which is incorporated herein by reference. The DNA sequence of U1 snRNP-associated 70K was obtained from GenBank (X04654) and translated into the amino acid sequence. The 614 amino acid sequence, rather than the shorter published 438 amino acid sequence, was used for the reasons discussed by Douvas and Sobelman, 1991. The shorter sequence lacks the extreme amino terminus, which is present in the longer sequence. However, this omission does not affect the overall homology between 70K and gp120/41.

Homologous sequences were defined as sequences of at least ten amino acids having at least 50% amino acid identity or as sequences of at least four amino acids having at least 75% identity, with identical amino acid sequences occurring in the same order in each homolog. The probabilities of such matches occurring at random are 1 in 1.6×10^6 and 1 in 1.3×10^5 , respectively. Consensus binding sequences (CBS's) were identified by visual inspection. Other regions of homology were identified by dot-matrix plot (window size 10, matches 5, score 100, ktup 2, speed 1) using the GenePro routine (Riverside Scientific; Riverside, CA) and a VAX/VMS computer. Extensive analysis of 41 control proteins (11,743 amino acids) also was performed to confirm the significance of the identified homologous regions (see Douvas and Sobelman, 1991).

Regions of homology shared between 70K and gp120/41 are shown in Figure 1.A. The large number of homologous regions (25) is apparent and involves 206 amino acids of 70K (33.5% of the amino acid sequence). One region of homology is the eight amino acid binding site of 70K for U1 RNA, which is contained in domain B (see rectangle at positions 322 to 329). In fact, this eight amino acid sequence is not an exact sequence but is a

- family of consensus binding sequences (CBS's) that occur in several nuclear U1 RNA binding proteins (Figure 1.B.). The CBS contains two invariant amino acids, G and F, and two nearly invariant amino acids, A and V, as indicated.
- 5 Although the V3 domain shown in Figure 1.B. was obtained from HIV-1 IIIB, the GRAFXT (SEQ ID NO: 26) configuration also occurs in strains MN, SF2 and SC.

Hydrophilic sequences in 70K, including the repeating RDRDR (SEQ ID NO: 16) motif, are homologous 10 primarily to gp41 (Figure 1.A.). These regions of homology include a 19 amino acid sequence, which begins at position 513 in 70K and position 732 in gp41, as well as three additional sequences in 70K (see Table 1, sequence 5). This sequence in gp41 is a major target of neutralizing 15 antibodies in individuals vaccinated with recombinant gp160 (Pincus et al., J. Clin. Invest. 91:1987-1996 (1993)).

The congruence between framework sequences in the U1 RNA CBS and conserved amino acids in V3 (Figure 1.B.) indicates that ligands that bind to the CBS also may bind 20 specifically to V3 and, therefore, can be immunologically homologous amino acid sequences. The immunologically homologous regions shared between 70K and the V3 loop involve a major cluster of neutralizing determinants for HIV antibodies. Two amino acid sequences of 70K align with 25 the V3 sequence (Figure 2). One sequence, which spans domain A, is immunologically homologous to a sequence at the amino terminus of V3. In addition, the CBS from domain B (sequence a) is immunologically homologous to a sequence near the V3 apex. Figure 2 also shows the CBS's of U1 RNA 30 binding polypeptides A and B1 (sequences b and c).

Overlapping neutralizing domains that have been identified in V3 are shown as solid lines around the loop in Figure 2. Synthetic peptides were used in direct and competition ELISA's to show that a broad region of 24 amino

acids (line 1) contains major neutralizing epitopes recognized by HIV-1-infected human sera (Rusche et al., Proc. Natl. Acad. Sci., USA 85:3198-3202 (1988)). In addition, broadly neutralizing monoclonal antibodies 5 identify a dominant neutralizing region (line 2; Durda et al., AIDS Res. Hum. Retroviruses 6:1115-1123 (1990)). Furthermore, a sequence largely overlapping line 2 (line 3) reacted with and blocked neutralization by a large panel of HIV-infected sera (Broliden et al., Proc. Natl. Acad. Sci., USA 89:461-465 (1992)). Sequences delineated by lines 4 10 and 5 reacted with and blocked neutralization by polyclonal and monoclonal anti-HIV antibodies, respectively (Javaherian et al., Proc. Natl. Acad. Sci., USA 86:6768-6772 (1989); Laman et al., Virology 66:1823-1831 (1992)). 15 At the amino terminus of the V3 loop, the region delineated by line 6 reacted with type-specific neutralizing HIV antibodies (Kenealy et al., AIDS Res. Hum. Retroviruses 5:173-182 (1989)).

The superposition of the six lines reveals that 20 the major neutralizing V3 epitope cluster coincides with sequences in the two immunodominant domains A and B of 70K and that immunologically homologous regions of 70K involve 50% of the V3 loop. Fine mapping of the apex of the V3 loop using human monoclonal antibodies further emphasizes 25 the conserved nature of key immunologically homologous sequences. Two short overlapping epitopes, GPGR (SEQ ID NO: 30) and GRAF (SEQ ID NO: 31; lines 7 and 7'), are present in divergent strains, including MN and IIIB, and are the targets of broadly neutralizing antibodies (Gorny 30 et al., Proc. Natl. Acad. Sci., USA 88:3238-3242 (1991); Gorny et al., J. Immunol. 150:635-643 (1993)). An additional conserved segment (line 8) also is the target of broadly neutralizing antibodies and is continuous with the GPGR epitope in MN, but not in IIIB.

Discontinuous and conformationally sensitive epitopes are important in the neutralizing C4 cluster of epitopes that involve the CD4-binding site of gp120, but less so in the V3 cluster. The linear V3 epitope 5 delineated by lines 7 and 7', which are targets of broadly neutralizing antibodies, are congruent with highly conserved amino acids in the U1 RNA-binding site of 70K and related splicing proteins. In 70K, as in other nuclear autoimmune antigens, the immunodominant sites also are the 10 functionally critical sites and virtually all anti-RNP antibodies inhibit RNA splicing.

EXAMPLE II

Crossreactivity of 70K and HIV-1 gp120/41

This example demonstrates that antiserum obtained 15 from HIV-1⁺ individuals can react with 70K and anti-RNP antibodies can react with gp120/41.

Anti-RNP sera were obtained from MCTD patients treated in the outpatient Rheumatology Clinic of the University of Southern California Health Sciences Center 20 (Los Angeles, CA) and were confirmed positive for anti-nuclear antibody (ANA) as determined by immunofluorescence and positive for anti-RNP as determined by double diffusion. Six donors having a clinical diagnosis of Sjögren's syndrome were confirmed to be SS-A/Ro antibody 25 positive. Normal sera were obtained from institutional personnel and were confirmed ANA negative. Sera from HIV-1 infected donors were obtained from the University of Southern California Health Sciences Center and were confirmed HIV⁺ by western blotting using a kit obtained from 30 Organon Teknika (Durham, NC). The immunoassay results presented in Figure 3 and Figure 4 were confirmed by isolating IgG from some anti-RNP sera and some control sera by ammonium sulfate fractionation and DEAE chromatography as described Douvas (1982).

Recombinant HIV-1 gp120 (Du Pont; Boston, MA), V3 IIIIB (Sigma; St. Louis, MO) and gp41 and V3 MN (ABT; Cambridge, MA) were purchased. Partially purified 70K for western blot analysis was isolated from rat liver 5 (Pelfreeze; Rogers, AK) using the nuclear fractionation and antibody affinity chromatography method described by Douvas et al. (1979) and Douvas (1982).

ELISA assays were performed essentially as described by Crow et al., Cell. Immunol. 121:99-112 (1989), 10 which is incorporated herein by reference. Briefly, saturating concentrations of antigen were adsorbed to plastic microtiter plates for 12 hr at 4°C, then the plates were washed and unreacted sites were blocked with 1% bovine serum albumin-phosphate-buffered saline (BSA/PBS; pH 7.5). 15 Sera were diluted 1:100 in 0.1% BSA/PBS and added to the appropriate wells. Samples were incubated at 4 °C overnight. Following incubation, horseradish peroxidase (HRP)-conjugated goat anti-human immunoglobulin (Ig) (Zymed, San Francisco, CA) was diluted 1:1000 and added to 20 the sample. Incubation was continued for 1 hr at room temperature and bound antibody was identified using o-phenyldiamine. Optical densities were determined at 490 nm using an automated ELISA reader.

Electrophoresis was performed using 10% 25 polyacrylamide gels and western blot analysis was performed as described by Towbin et al., Proc. Natl. Acad. Sci., USA 76:4350-4354 (1979), which is incorporated herein by reference. Sera were diluted 1:250 for western blot analysis. Blots were developed using HRP-conjugated goat 30 anti-human Ig (1:3000 dilution) as a second antibody (Tago; Concord, CA).

The crossreactivity of 70K and gp120/41 that was predicted by the amino acid sequence homology analyses (Figures 1 and 2) was confirmed using ELISA to compare the

reactivity of twelve HIV-1-positive sera, ten anti-RNP sera, a rheumatoid control group of six Sjögren's syndrome sera (SS) and eight normal sera (NL) to HIV-1 antigens (Figure 3). The HIV-1 antigens used were recombinant 5 gp120, V3 (IIIB) and gp41.

The mean reactivity to gp120 was highest for HIV sera (0.67 OD₄₉₀), as compared to RNP (0.13), SS (0.14) and NL (0.03) sera (Figure 3.A.). Similarly, HIV sera (0.5) had the highest reactivity against gp41 as compared to RNP 10 (0.26), SS (0.15) and NL (0.09) sera (Figure 3.C.). In contrast, RNP sera had the highest reactivity (0.29) against the V3 loop as compared to HIV (0.26), SS (0.16) and NL (0.09) sera (Figure 3.B.).

The results also indicate that the reactivity of 15 the SS group of sera are higher than normal but are essentially the same for all three HIV antigens. In addition, the SS sera react with the Ro/SSA antigen, which has no significant structural homology to gp120/41. Thus, the SS sera demonstrate the well known general hyper- 20 reactivity of autoimmune sera, but no evidence of epitope specificity based on structural homology.

The results indicate that HIV⁺ sera have the highest reactivity to gp120, which contains a number of additional epitopes, including those in the C4 cluster that 25 are not homologous to 70K. In contrast, the anti-RNP sera demonstrate a greater than two-fold higher reactivity to V3 than to the entire gp120 molecule and a reactivity to V3 that is equivalent (slightly higher) to the reactivity of HIV⁺ sera. This result is consistent with the higher 30 concentration of immunologically homologous regions in the V3 loop that are congruent with dominant epitopes in 70K (see Figure 2). Anti-RNP sera also are two-fold more reactive to gp41 than to gp120. As indicated in Figure 1, a large proportion of the gp41 immunologically homologous

- regions corresponds to hydrophilic sequences and motifs such as the repeating RDRDR (SEQ ID NO: 16) sequence, which are epitopes in 70K and which account for a large proportion of the hydrophilic carboxy terminus of 70K.
- 5 These include a 19 amino acid sequence, which begins at position 732 of gp41 (see, also, Table 1).

The reactivity of HIV⁺ and anti-RNP sera against the V3 loop of two divergent HIV-1 strains, IIIb and MN, also was compared by ELISA. A marked strain specificity of 10 HIV⁺ sera for MN over IIIb was observed (Figure 4.A.; mean values of 0.536 and 0.144, respectively, or a 3.7-fold preference for MN). Figure 4.A. also shows the reactivities of a normal serum (NL) for IIIb (0.02) and MN (0.03). The anti-RNP sera had only a 33% greater 15 reactivity against IIIb than MN (Figure 4.B.). These results provide experimental support for the homology analyses, which revealed that conserved invariant sequences in the CBS of 70K and related U1 RNA-binding proteins were immunologically homologous to conserved amino acid 20 sequences in the V3 loop (Figures 1 and 2).

Anti-RNP autoantibodies are predominantly of the IgG isotype. To determine the isotype of the crossreactive antibodies detected by ELISA assays (Figures 3 and 4), heat-inactivated sera were subjected to ammonium sulfate 25 fractionation and ion-exchange chromatography as described by Douvas (1982). The results indicated that 95% of the reactivity in each serum examined was due to IgG antibodies and that less than 5% was due to IgM. Moreover, when comparisons shown in Figure 4 were repeated using purified 30 IgG, the results again showed marked preference for MN by HIV⁺ sera and no substantial selectivity by the anti-RNP autoantibodies.

Western blot analysis was used to determine whether sera from HIV-infected individuals recognize 70K

epitopes. 70K was partially purified from nuclei as described by Douvas (1982). All ten HIV⁺ sera reacted with 70K, as well as a breakdown product of 70K (Figure 5). Eight of the HIV⁺ sera reacted strongly with 70K and one serum (lane 7) consistently reacted more strongly than anti-RNP sera (lanes 12 to 14). The strong crossreactivity of HIV sera with 70K indicate that the T cells of HIV-infected individuals can react with a 70K epitope.

Table 1 compares the immunologically homologous regions of 70K with gp120/41 epitopes that are associated with viral neutralization, which is a measure of immunoprotection, and epitopes that are associated with deleterious effects. Although the gp120/41 sequences that are associated with enhancement of infection, anergy, or immunosuppression include some sequences that are homologous to class I and class II MHC molecules, none of the sequences were homologous to 70K (Table 1.B.).

In contrast, neutralizing epitopes of gp120/41 aligned with a total of eleven non-overlapping 70K immunologically homologous regions. In particular, the sequences 1 and 5 include two major antigenic motifs of 70K, ERKR (SEQ ID NO: 32) and RDRDR (SEQ ID NO: 16). The RDRDR (SEQ ID NO: 16) motif is contained in a long 70K sequence that shares extensive homology to sequence 5 of gp41. Sequence 5 of gp41 is considered a dominant antigen both as a site of reactivity for HIV-infected sera and as a target of antibodies from volunteers vaccinated with gp160. The homology between the 70K CBS and sequence 2 was discussed above.

Sequence 3 of gp120, RLGGGDMR, is immunologically homologous to three permutations of a 70K sequence, GGGDM (SEQ ID NO: 33), RLGGG (SEQ ID NO: 34) and LRGGG (SEQ ID NO: 35). Antibodies to peptides containing this sequence are neutralizing, although at low titers. Deletion

mutation of gp120 in the GGG triplet and beyond abolishes its ability to bind to CD4⁺ cells (Kowalski et al., Science 237:1351-1355 (1987)). Sequence 4, a neutralizing epitope in gp41, which is immunologically homologous to a sequence 5 near the amino terminus of 70K, also is contained in a peptide that directly inhibits HIV-1 replication (Surowy et al., 1989).

The results presented above indicate that (1) anti-RNP antibodies cross-react with the V3 loop at titers 10 equivalent to those of HIV⁺ sera (Figure 3); (2) major neutralizing epitopes in V3 coincide with dominant epitopes in 70K, including its U1 RNA CBS (Fig 2); (3) hydrophilic epitopes in gp41 are immunologically homologous to antigenic, hydrophilic motifs in 70K (Figure 1 and Table 15 1); (4) autoimmune disease control antibodies from Sjögren's syndrome lack selectivity for both V3 and gp41 (Figure 3); and (5) HIV antibodies cross-react with 70K (Figure 5).

EXAMPLE III

20 Diagnostic Skin Test for HIV-1 Infection

This example provides the method for performing the skin test and evaluating the results of the test.

Approximately 0.1 ml of a composition comprising 25 an amino acid sequence that is immunologically homologous to an epitope present on HIV-1 and can stimulate an immune response and a pharmacologically acceptable carrier is injected intradermally on the flexor or dorsal surface of the forearm, about 4 inches below the elbow. Prior to injection, the site should be cleansed with a solution of 30 70% ethyl alcohol. A disposable syringe and needle can be used for injection and a separate sterile unit is used for each person tested.

The point of the needle is inserted into the most superficial layers of the skin with the needle bevel pointing upward. Injection of the composition results in the formation of a pale bleb 6-10 mm in size, which is 5 quickly absorbed. If no bleb forms, the injection was likely delivered subcutaneously and the test should be repeated immediately at another site at least 5 cm removed. Similarly, if the composition leaks from the injection site, the test should be repeated.

10 Evidence of an immune response is determined between 48 and 72 hr after injection of the composition and at any additional times prescribed. The reaction size is calculated as one-half the sum of the perpendicular diameters. Reactions greater than 5 mm are considered 15 "positive." Care should be taken to determine that a "negative" reaction is not erroneous due, for example, to a non-specific suppressor such as non-HIV viral infections, live virus vaccines, prior administration of corticosteroids or malnutrition.

20 Although the invention has been described with reference to the above examples, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: University of Southern California
- (ii) TITLE OF INVENTION: Methods to Diagnose and Treat HIV-1 Infection
- (iii) NUMBER OF SEQUENCES: 66
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Campbell and Flores
 - (B) STREET: 4370 La Jolla Village Drive, Suite 700
 - (C) CITY: San Diego
 - (D) STATE: California
 - (E) COUNTRY: USA
 - (F) ZIP: 92122
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE: 13-MAR-1995
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Imbra, Richard J.
 - (B) REGISTRATION NUMBER: 37,643
 - (C) REFERENCE/DOCKET NUMBER: FP-SI 1394
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (619) 535-9001
 - (B) TELEFAX: (619) 535-8949

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
Gly Glu Arg Leu Asp Arg Arg Lys Glu Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Leu Ile Glu Asp Gln Gln Gln Arg Gln Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Pro Gly Arg Ala Ala Ser Ser Ala Gly
1 5

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Gly Leu Val Arg Ser Ser Ser Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Pro Arg Ala Ser Gly Gln Thr Pro Glu Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Thr Arg Glu Glu Arg Met Glu Arg Lys Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Leu Lys Met Trp Asp Pro His Asn Asp Pro Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gly Tyr Ala Phe Ile Glu Tyr Glu
1 5

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Pro Arg Arg Leu Gly Gly Gly Leu
1 5

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 11 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gly Ala Asp Val Asn Ile Arg His Ser Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Arg Pro Gly Asp Ser Pro Leu Pro His Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Glu Arg Asp Lys Glu Arg Arg Arg Ser Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asp Lys Asp Arg Asp Arg Lys Arg Arg Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 11 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Glu Glu Leu Arg Gly Gly Gly Gly Asp Met Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 19 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gly Pro Asp Gly Pro Asp Gly Pro Glu Glu Lys Gly Arg Asp Arg Asp
1 5 10 15
Arg Glu Arg

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Arg Asp Arg Asp Arg
1 5

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Arg Gly Gly Gly Gly Gln Asp Asn Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gly Phe Gln Phe Val Thr Phe Asp
1 5

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gly Phe Ala Phe Val Thr Phe Asp
1 5

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gly Gln Ala Phe Val Ile Phe Lys
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 614 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Gly Thr Ile Ser Gly Gly Gly Ser Asn Ala Ala Thr Arg Gln
1 5 10 15

Val Gly Cys Ala Pro Ser Gly Arg Pro Ser Thr Arg Pro Ser Gly Thr
20 25 30

Ala Ile Arg Ala Arg Pro Val Ala Ser Val Lys Pro Ile Asp Glu Gly
35 40 45

Leu Ala Glu Val Arg Val Ile Glu Asp Glu Ala Ile Gly Ile Glu Gly
50 55 60

Glu Arg Leu Asp Arg Arg Lys Glu Arg Arg Gln Glu Ala Leu Ile
65 70 75 80

Glu Asp Gln Gln Arg Gln Arg Arg Trp Pro Gly Leu Pro Ala Ala
85 90 95

Arg Pro Gly Arg Ala Ala Ser Ser Ala Gly Ile Gly Gly Arg Gln Gly
100 105 110

Leu Leu Ala Arg Gly Thr Leu Trp Trp Leu Ser Ser Gly Leu Val Arg
115 120 125

Ser Ser Ser Gly Arg Arg Asn Gln Thr Asp Val Asp Ala Pro Gly Val
130 135 140

Glu Ala Glu Ala Gly Val Val Val Ala Glu Gly Leu Pro Gln Pro Pro
145 150 155 160

Arg Ala Ser Gly Gln Thr Pro Glu Arg Gly Gly Ala Thr Arg Leu Gly
165 170 175

Lys Met Thr Gln Phe Leu Pro Pro Asn Leu Leu Ala Leu Phe Ala Pro
180 185 190

Arg Asp Pro Ile Pro Tyr Leu Pro Pro Leu Glu Lys Leu Pro His Glu
195 200 205

Lys His His Asn Gln Pro Tyr Cys Gly Ile Ala Pro Tyr Ile Arg Glu
210 215 220

Phe Glu Asp Pro Arg Asp Ala Pro Pro Thr Arg Ala Glu Thr Arg
225 230 235 240

Glu Glu Arg Met Glu Arg Lys Arg Arg Glu Lys Ile Glu Arg Arg Gln
245 250 255

Gln Glu Val Glu Thr Glu Leu Lys Met Trp Asp Pro His Asn Asp Pro
260 265 270

Asn Ala Gln Gly Asp Ala Phe Lys Thr Leu Phe Val Ala Arg Val Asn
275 280 285

Tyr Asp Thr Thr Glu Ser Lys Leu Arg Arg Glu Phe Val Tyr Gly
290 295 300

Pro Ile Lys Arg Ile His Met Val Tyr Ser Lys Arg Ser Gly Lys Pro
305 310 315 320

Arg Gly Tyr Ala Phe Ile Glu Tyr Glu His Glu Arg Asp Met His Ser
325 330 335

Ala Tyr Lys His Ala Asp Gly Lys Ile Asp Gly Arg Arg Val Leu
340 345 350

Val Asp Val Glu Arg Gly Arg Thr Val Lys Gly Trp Arg Pro Arg Arg
355 360 365

Leu Gly Gly Leu Gly Gly Thr Arg Arg Gly Gly Ala Asp Val Asn
370 375 380

Ile Arg His Ser Gly Arg Asp Asp Thr Ser Arg Tyr Asp Glu Arg Pro
385 390 395 400

Gly Pro Ser Pro Leu Pro His Arg Asp Arg Asp Arg Asp Arg Glu Arg
405 410 415

Glu Arg Arg Glu Arg Ser Arg Glu Arg Asp Lys Glu Arg Glu Arg Arg
420 425 430

Arg Ser Arg Ser Arg Asp Arg Arg Arg Ser Arg Asp Lys
435 440 445

Glu Glu Arg Arg Arg Ser Arg Glu Arg Ser Lys Asp Lys Asp Arg Asp
450 455 460

Arg Lys Arg Arg Ser Ser Arg Glu Arg Ala Arg Arg Glu Arg
465 470 475 480

Glu Arg Lys Glu Glu Leu Arg Gly Gly Asp Met Ala Glu Pro
485 490 495

Ser Glu Ala Gly Asp Ala Pro Pro Asp Asp Gly Pro Pro Gly Glu Leu
500 505 510

Gly Pro Asp Gly Pro Asp Gly Pro Glu Glu Lys Gly Arg Asp Arg Asp
515 520 525

Arg Glu Arg Arg Arg Ser His Arg Ser Glu Arg Arg Arg Asp
530 535 540

Arg Asp Arg Asp Arg Asp Arg Asp Arg Glu His Lys Arg Gly Glu Arg
545 550 555 560

Gly Ser Glu Arg Gly Arg Asp Glu Ala Arg Gly Gly Gly Gly Gln
565 570 575

Asp Asn Gly Leu Glu Gly Leu Gly Asn Asp Ser Arg Asp Met Tyr Met
580 585 590

Glu Ser Glu Gly Gly Asp Gly Tyr Leu Ala Pro Glu Asn Gly Tyr Leu
595 600 605

Met Glu Ala Ala Pro Glu
610

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gly Tyr Ala Phe
1

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Glu Arg Pro Glu Glu Arg Glu Glu Arg Arg Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Glu Arg Lys Arg Arg
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gly Arg Ala Phe Val Thr Ile Gly
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /note= "Xaa = any one independently selected amino acid."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gly	Arg	Ala	Phe	Xaa	Thr
1				5	

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Gly	Arg	Ala	Phe	Val	Thr
1				5	

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gly	Arg	Ala	Phe	Tyr	Thr
1				5	

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gly Pro Gly Arg
1

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Gly Arg Ala Phe
1

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Glu Arg Lys Arg
1

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gly Gly Gly Asp Met
1 5

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Arg Leu Gly Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Leu Arg Gly Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 220 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Gly Ile Glu Gly Glu Arg Leu Asp Arg Arg Lys Glu Arg Arg Arg Gln
1 5 10 15

Gln Glu Ala Leu Ile Glu Asp Gln Gln Arg Gln Arg Pro Gly Arg
20 25 30

Ala Ala Ser Ser Ala Gly Ile Gly Gly Arg Gln Gly Leu Leu Ser Gly
35 40 45

Leu Val Arg Ser Ser Ser Gly Arg Pro Arg Ala Ser Gly Gln Thr Pro
50 55 60

Glu Arg Thr Arg Glu Glu Arg Met Glu Arg Lys Arg Leu Lys Met Trp
65 70 75 80

Asp Pro His Asn Asp Pro Asn Ser Lys Leu Arg Arg Glu Phe Glu Val
85 90 95

Tyr Gly Tyr Ala Phe Ile Glu Tyr Glu His Pro Arg Arg Leu Gly Gly
100 105 110

Gly Leu Gly Gly Thr Arg Arg Gly Gly Ala Asp Val Asn Ile Arg His
115 120 125

Ser Gly Arg Arg Pro Gly Asp Ser Pro Leu Pro His Arg Asp Arg Asp
130 135 140

Arg Asp Arg Glu Arg Asp Lys Glu Arg Arg Arg Ser Arg Asp Lys Asp
145 150 155 160

Arg Asp Arg Lys Arg Arg Ser Ser Arg Glu Glu Leu Arg Gly Gly Gly
165 170 175

Gly Asp Met Ala Gly Pro Asp Gly Pro Asp Gly Pro Glu Glu Lys Gly
180 185 190

Arg Asp Arg Asp Arg Glu Arg Arg Asp Arg Asp Arg Asp Arg Asp Arg
195 200 205

Asp Arg Arg Gly Gly Gly Gln Asp Asn Gly
210 215 220

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Gly Ile Glu Glu Glu Gly Glu Arg Asp Arg Asp Arg Ser Ile Arg Leu
1 5 10 15

Ile Glu Glu Ser Gln Asn Gln Gln Glu Pro Gly Arg Ala Phe Val Thr
20 25 30

Ile Gly Gln Leu Leu Gly Ser Gln Ile Arg Cys Ser Ser Asn Ile
35 40 45

Pro Arg Arg Ile Arg Gln Gly Leu Glu Arg Thr Arg Pro Asn Asn Asn
50 55 60

Thr Arg Lys Arg Leu Lys Cys Thr Asp Leu Lys Asn Asp Thr Asn Ser
65 70 75 80

Lys Leu Arg Glu Gln Phe Gly Asn Asn Gly Arg Ala Phe Val Thr Ile
85 90 95

Gly Lys Pro Arg Arg Ile Arg Gln Gly Leu Gly Ala Cys Arg Ala Ile
100 105 110

Arg His Ile Pro Arg Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Glu
115 120 125

Arg Asp Arg Asp Asp Arg Ser Ile Arg Glu Ile Phe Arg Leu Gly Gly
130 135 140

Gly Asp Met Arg Gly Pro Asp Arg Pro Glu Gly Ile Glu Glu Glu Gly
145 150 155 160

Gly Glu Arg Asp Arg Asp Arg Glu Arg Asp Arg Asp Arg Leu Gly Gly
165 170 175

Gly Asp Met Arg Asp Asn Trp
180

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Ser Ser Ser Gly Arg
1 5

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Thr Arg Asp Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Glu Arg Asp Arg Asp Arg
1 5

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Glu Arg Asp Arg Asp Ser Arg Ser Ile Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Glu Leu Leu Gly Arg Arg Gly Trp Glu Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Lys Lys Arg Gly Phe Gln Phe Val Thr Phe Asp Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Lys Lys Arg Gly Phe Ala Phe Val Thr Phe Asp Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Lys Pro Arg Gly Tyr Ala Phe Ile Glu Tyr Glu His
1 5 10

(2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Lys Ala Arg Gly Gln Ala Phe Val Ile Phe Lys Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Lys Met Arg Gly Gln Ala Phe Val Ile Phe Lys Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Arg Pro Arg Gly Val Ala Phe Val Arg Tyr Asn Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Arg Leu Gly Gly Gly Asp Met Arg
1 5

(2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Thr Arg Pro Asn Asn Asn Thr Arg Lys Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Pro Gly Arg Ala
1

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Gly Tyr Ala Phe Ile Glu Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Leu Ile Glu Asp Gln Gln Gln Arg Gln
1 5

(2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Gly Pro Asp Arg Pro Glu Gly Ile Glu Glu Glu Gly Gly Glu Arg Asp
1 5 10 15
Arg Asp Arg

(2) INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Gly Pro Asp Gly Pro Asp Gly Pro Glu Glu Lys Gly Arg Asp Arg Asp
1 5 10 15
Arg Glu Arg

(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Arg Asp Arg Asp Arg Asp Arg
1 5

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Asp Arg Asp Arg
1

(2) INFORMATION FOR SEQ ID NO:61:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Arg Ile Leu Ala Val Glu Arg Tyr Leu Lys Asp Gln Gln Leu Gly Ile
1 5 10 15
Trp Gly Cys Ser Gly Lys Leu Leu Cys
20 25

(2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Ser Leu Glu Gln Ala Gln Ile Gln Gln Glu Lys Asn Glu Gln Glu Leu
1 5 10 15
Leu Lys Leu

(2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Glu Gly Thr Asp Arg Val Ile
1 5

(2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:65:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Gly Ser Thr Met Gly Ala Ala Ser Met Thr Leu Thr Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:66:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Gln Glu Leu Lys Asn Ser Ala Val Ser Leu
1 5 10

We claim:

1. A composition having an amino acid sequence of a 70K autoantigen or a CENP-B autoantigen, characterized in that said amino acid sequence is immunologically homologous to HIV-1.

2. A composition having an amino acid sequence of an autoantigen, characterized in that said amino acid sequence is immunologically homologous to HIV-1 gp120/41.

3. The composition of claim 2, characterized in 10 that said autoantigen is 70K and said immunologically homologous amino acid sequence is:

GERLDRRKER (SEQ ID NO: 1);
LIEDQQQRQR (SEQ ID NO: 2);
PGRAASSAG (SEQ ID NO: 3);
15 SGLVRSSSGR (SEQ ID NO: 4);
PRASGQTPER (SEQ ID NO: 5);
TREERMERKR (SEQ ID NO: 6);
LKMWDPHNDPN (SEQ ID NO: 7);
GYAFIEYE (SEQ ID NO: 8);
20 PRRLGGGL (SEQ ID NO: 9);
GADVNIRHSGR (SEQ ID NO: 10);
RPGDSPPLPHR (SEQ ID NO: 11);
ERDKERRRSR (SEQ ID NO: 12);
DKDRDRKRRS (SEQ ID NO: 13);
25 EELRGGGDMA (SEQ ID NO: 14);
GPDGPDGPEEKGRDRDRER (SEQ ID NO: 15);
RDRDR (SEQ ID NO: 16) or
RGGGGGQDNG (SEQ ID NO: 17).

4. The composition of claim 2, characterized in that said immunologically homologous amino acid sequence is:

5 GFQFVTFD (SEQ ID NO: 18);
GFAFVTFD (SEQ ID NO: 19); or
GQAFVIFK (SEQ ID NO: 20).

5. The composition as defined in any of claims 1 to 4, characterized in that said immunologically homologous amino acid sequence is immunogenic.

10 6. A pharmaceutical composition containing a pharmaceutically acceptable carrier and an autoantigen, characterized in that the autoantigen is a composition as defined in any of claims 1 to 5.

15 7. The pharmaceutical composition of claim 6, characterized in that said autoantigen is 70K as shown in Figure 6 (SEQ ID NO: 21).

8. The composition of any of claims 1 to 7 for use in a method of diagnosing HIV-1 infection in a human subject.

20 9. The composition of claim 8, characterized in that said method is a skin test.

10. The composition of claim 8, characterized in that said method is performed *in vitro*.

25 11. A kit for the diagnosis of HIV-1 infection in a subject suspected of having an HIV-1 infection, characterized in that said kit contains a solid support having attached thereto any of the compositions of claims 1 to 7, a control antibody, which binds said composition, and a detectable moiety.

12. The composition of any of claims 1 to 7 for use in a method for therapeutic treatment of a human subject.

13. The composition of any of claim 13,
5 characterized in that said therapeutic treatment is a method of stimulating an immune response against HIV-1.

FIGURE 1

GP120/41 61 GIE//GERLDRRKERRRQUEALIEDQQQRQR 98 PGRASSAGIGGRQGLL 124 SGLVRSSSGR 159 PRASGOTPER
 NP 70K 732 GIEEEEGERDRRSIR 645 LIEESQNQQE 319 PGRAFVTIG 595QLLG 440SGQIRCSSNI 844 PRRIRQGLER
 GP120/41

239 TREERMERKR 263 LKMWDPHNDPN 294 SKLRFREFEVY 322 **GYAFIYEY**
 303 TRPHINTRKR 129 LKCTDLKNDTN 147 SKLREQFGNN 321**GRAFVTICK**

245 —— 249 276 ——
 A B

739 ERDRUR 739 ERDRDSRSIR 79) ELLGRIGWEA
 399 RPGDPLPHRDRDR 424 ERDKERRRSR 460 DKDRDRKRRSSR 484 EELRGGGDMA 513 GPDGPDGPEEKGRDRDR
 709 RQGYSPLSFQ 734 ERDRDDRSIR 466 EIFRLGGGDMR 732 CPDRPEGIEEGGERDRDI:

740 RDRDR 542 RDRDRDRDR 570 RGGGGGQDNG
 739 ERDRDR 470 LGGGDMRDNW

B

HIV-1 V, ... RGPG[A]FV[TICK]
 hnRNP A2/B1 ... KKRC[FQ]FVTFDD
 hnRNP A1 ... KKRC[FQ]FVTFDD
 U,snRNP 70K ... KPREYAFTEYEU
 U,snRNP A ... KARGOAFVIFRE
 U,snRNP B1 ... KMRCGAFVIFRE
 fly **8x1#2** ... RPRG[A]FVRYNK

FIGURE 2

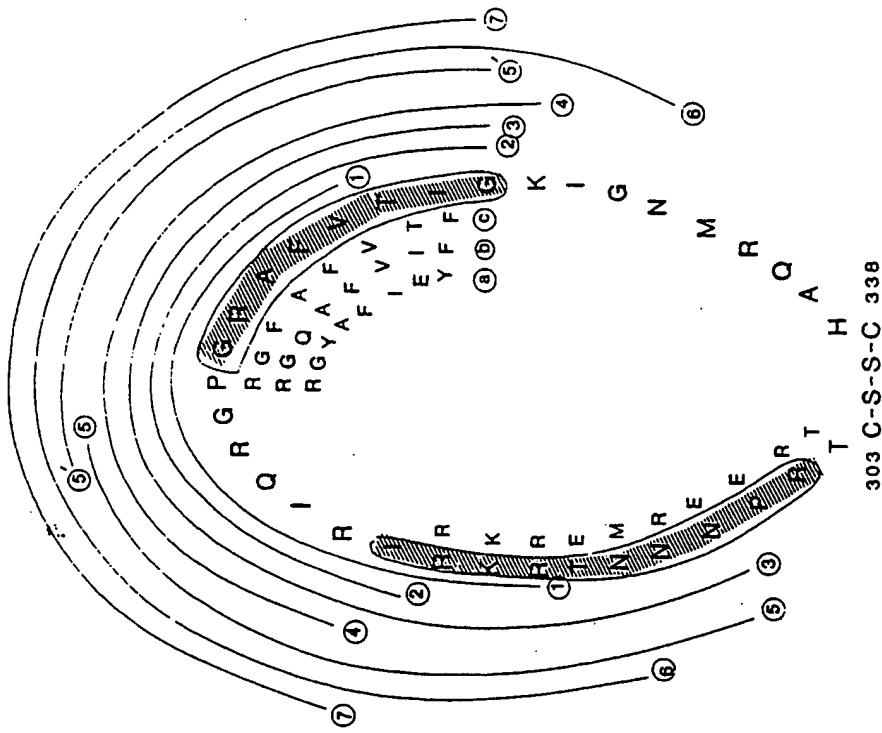


FIGURE 3

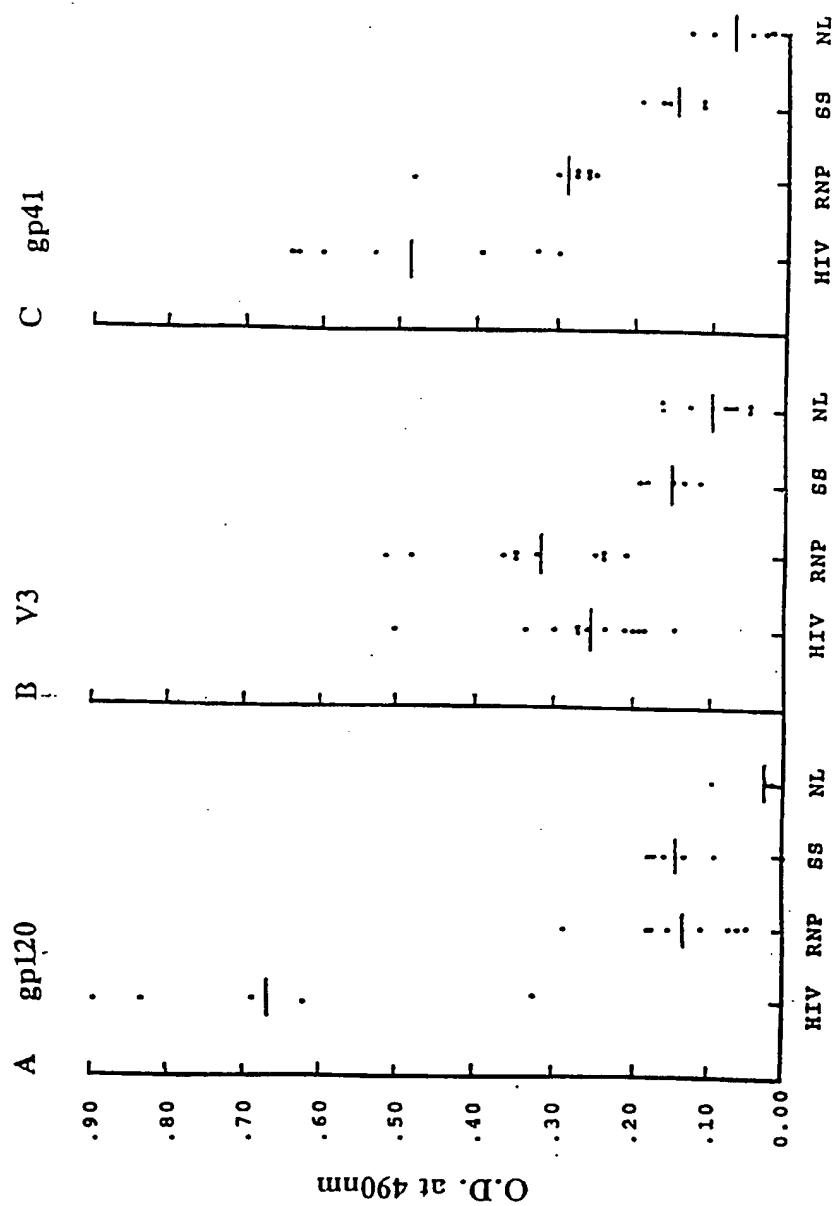


FIGURE 4

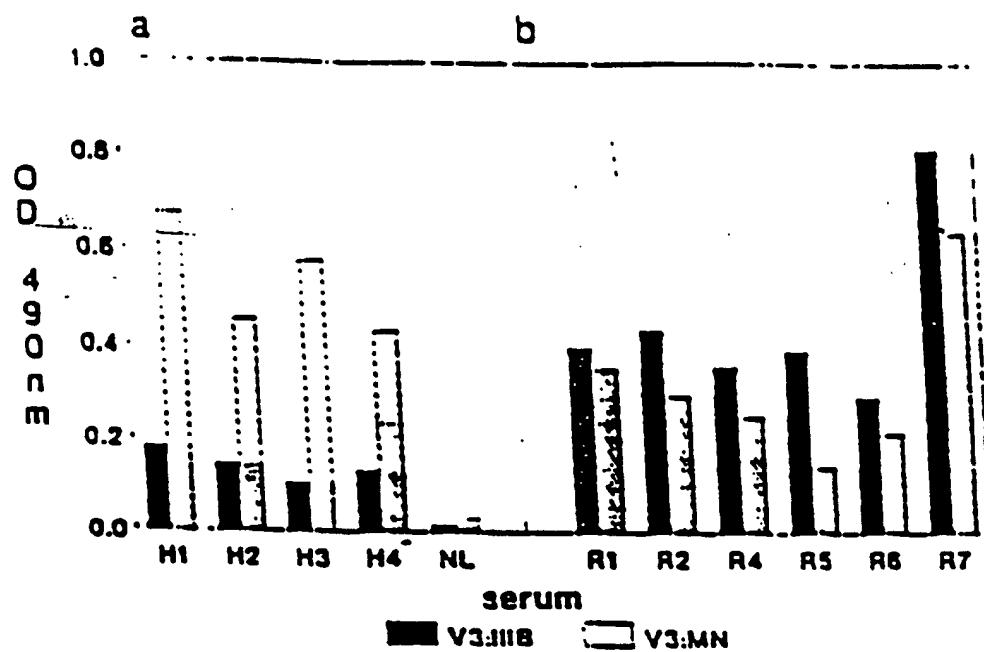


FIGURE 5

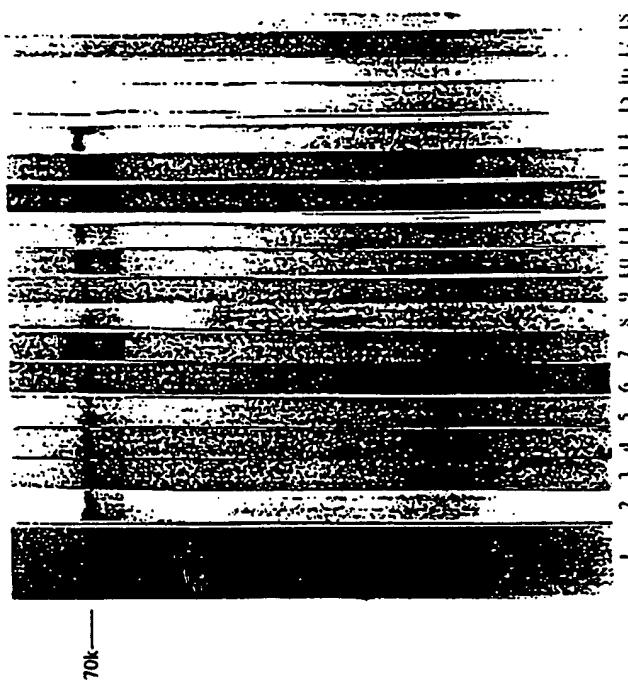


FIGURE 6
AMINO ACID SEQUENCE OF 70K

	MGTISGGGGS	NAATRQVGCA	PSGRPSTRPS	GTAIRARPVA	SVKPIDEGLA
	EVRVIEDEAI	GIEGERLDRR	KERRQEALI	EDQQQRQRWW	PGLPAARPGR
5	AASSAGIGGR	QGLLARGTLW	WLSSGLV RSS	SGRRNQTDVD	APGV EAEAGV
	VVAEGLPQPP	RASGQTPERG	GATRLGKMTQ	FLFPNLLALE	APRDPPIP YLP
	PLEKLPHKH	HNQPYCGIAP	YIREFEDPRD	APPPTRAETR	EERMERKRRE
	KIERRQQEVE	TELKMWDPHN	DPNAQGDAFK	TLFVARVNYD	TTE SKLRREF
	EVYGPIKRIH	MVYSKRSGKP	RGYAFIEYEH	ERDMHSAYKH	ADGKKIDGRR
10	VLVDVERGRT	VKGWRPRRLG	GGGGT RRG	ADVNIHSGR	DDTSRYDERP
	GPSPLPHRDR	DRDRERERRE	RSRERDKERE	RRRSRSRD RR	RRSRSRDKEE
	RRRSRERSKD	KDRDRKRRSS	RSRERARRER	ERKEELRG GG	GDMAEPSEAG
	DAPPDDGPPG	ELGPDPGP	E EKGRDRDRE	RRRSHRSERE	RRDRDRDRD
	RDREHKRGER	GSERGRDEAR	GGGGQDNGL	EGLGNDSRDM	YMESEG GDGY
15	LAPENGYLME	AAPE			

INTERNATIONAL SEARCH REPORT

Int. Nat Application No PCT/US 95/03236
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A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C07K14/47 C07K14/16 A61K38/04 G01N33/569										
According to International Patent Classification (IPC) or to both national classification and IPC										
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 C07K A61K G01N										
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched										
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)										
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left; padding: 2px;">Category *</th> <th style="text-align: left; padding: 2px;">Citation of document, with indication, where appropriate, of the relevant passages</th> <th style="text-align: left; padding: 2px;">Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td style="padding: 2px;">X</td> <td style="padding: 2px;"> AIDS RESEARCH AND HUMAN RETROVIRUS, vol.10, no.3, March 1994, NEW YORK, USA pages 253 - 262 A DOUVAS & Y TAKEHANA 'Cross-reactivity between autoimmune anti-U1 snRNP antibodies and neutralizing epitopes of HIV-1 gp120/141' see the whole document --- PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol.88, no.14, 15 July 1991, WASHINGTON US pages 6328 - 6332 A DOUVAS & S SOBELMAN 'Multiple overlapping homologies between two rheumatoid antigens and immunosuppressive peptides' see the whole document --- -/-/ </td> <td style="padding: 2px;">1-13</td> </tr> <tr> <td style="padding: 2px;">X</td> <td style="padding: 2px;"></td> <td style="padding: 2px;">1-3,5-13</td> </tr> </tbody> </table>		Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X	AIDS RESEARCH AND HUMAN RETROVIRUS, vol.10, no.3, March 1994, NEW YORK, USA pages 253 - 262 A DOUVAS & Y TAKEHANA 'Cross-reactivity between autoimmune anti-U1 snRNP antibodies and neutralizing epitopes of HIV-1 gp120/141' see the whole document --- PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol.88, no.14, 15 July 1991, WASHINGTON US pages 6328 - 6332 A DOUVAS & S SOBELMAN 'Multiple overlapping homologies between two rheumatoid antigens and immunosuppressive peptides' see the whole document --- -/-/	1-13	X		1-3,5-13
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X		1-3,5-13								
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1 Date of the actual completion of the international search 3 July 1995										
Date of mailing of the international search report - 5. 07. 95										
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentdaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016										
Authorized officer Masturzo, P										

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Inte r nal Application No
PCT/US 95/03236

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>CELL, vol.51, no.2, 23 October 1987, CAMBRIDGE, MA US pages 211 - 220 C C QUERY & J D KEENE 'A human autoimmune protein associated with U1 RNA contains a region of homology that is cross-reactive with retroviral p30gag antigen' see the whole document</p> <p>---</p>	1-3,5-13
X	<p>EUR. J. IMMUNOL., vol.23, no.11, November 1993, WEINHEIM, GERMANY pages 2777 - 2781 C BERGMANN ET AL. 'An endogenously synthesized decamer peptide efficiently primes cytotoxic T cell specific for the HIV-1 envelope glycoprotein' see the whole document</p> <p>-----</p>	4
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